CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
para-DICHLOROBENZENE

SB 950-083, Tolerance # 50395, Chemical Code # 455

August 21, 1986
Revised 11/2/87, 7/18/88, 9/6/88, 1/20/89, 8/2/89, 1/2/92, 4/7/93, 12/27/93, 3/3/94, 1/27/95, 4/11/96, and 3/6/97

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effect
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, rat:	No data gap, possible adverse effect
Oncogenicity, mouse:	No data gap, possible adverse effect
Reproduction, rat:	No data gap, possible adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome mutation:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time†

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† Acceptable rat acute and subchronic neurotoxicity studies have been submitted. No adverse effects were noted.

Toxicology one-liners are attached

In one-liner headings below:
** indicates acceptable study.
Bold face indicates possible adverse effect;

Revised 9/88 by M. Silva; 1/89 by C. Aldous and J. Gee; 8/89 by G. Chernoff; 1/2/92 by P. Leung; 4/7/93, 12/27/93, 3/3/94, 1/27/95, 4/11/96, and 3/6/97 by C. Aldous.

All SB-950-mandated submissions in the DPR database as of 3/5/97 were considered for this revision. This includes all record numbers through 152815 (Document No. 50395-045).

Note: these pages contain summaries only. Individual worksheets may identify additional effects.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

CHRONIC AND COMBINED CHRONIC/ONCOGENICITY, RAT
(see also ONCOGENICITY, RAT section, below)

NOTE: Following receipt of an acceptable chronic oral dog study (Record No. 145808), and considering data in various chronic and subchronic rat studies previously submitted, DPR has considered that there is no further need to perform a repeat rat chronic study. (Aldous, 4/11/96).

NOTE: As of 12/27/93, para-dichlorobenzene is considered to indicate a "possible adverse effect" for oncogenicity (based on the NTP gavage study under the heading "Oncogenicity, Rat", below). The effect of concern is epithelial tumors of the kidneys of male rats. There is a dose-response relationship for an altered form of inclusion bodies, the normal form of which is regularly found in the renal tubular epithelia of male rats. The altered crystalline-like inclusion bodies are found in association with epithelial cell necrosis at intermediate doses, and eventually with the tumors at higher dose levels. Considerable evidence has been presented, consistent with the hypothesis that the syndrome is a male rat-specific response involving a2μ-globulin protein interactions. There are no analogous protein inclusions in humans, and the a2μ-globulin-like symptoms are not known to be relevant to humans in kidneys or elsewhere in the body. At present, only the tumor findings are considered as "possible adverse effects" under the "rat, oncogenicity" study category. The associated predisposing non-neoplastic lesions are not considered separately as "possible adverse chronic effects", because of the apparent relationship to the oncogenicity findings already flagged. Aldous, 12/27/93.

NOTE: Several 1-liners have noted that no ophthalmology was performed in the available rat chronic or oncogenicity studies. In the absence of available ophthalmology in long-term studies, it is noteworthy that an inhalation rat subchronic study (Record No. 131791) included ophthalmology examinations, and no untoward effects were identified. Aldous, 1/27/95, 4/11/96.

017 055701 Riley, R.A., et al., "Para-Dichlorobenzene: Long Term Inhalation Study in the Rat." (ICI Central Toxicology Lab., UK, 8/8/80) Wistar rats, 76-79/sex/group, exposed by inhalation to 0, 75, or 500 ppm of para-dichlorobenzene for 76 weeks followed by 36 weeks of recovery period. Interim kills at 26, 52, and 76 weeks, with terminal kills at weeks 109-112. Chronic NOEL = 75 ppm (LDT)-Organ (Liver, kidney, heart, lung) weight increases; Oncogenicity NOEL = 500 ppm (HDT). **NO ADVERSE EFFECT**. Previous reviews (Davis 8/14/86; Remsen 1/17/86) found "possible adverse effects" based on summary data (Records 043234, 043236, 043237, and 036703). **UNACCEPTABLE** as oncogenicity and/or chronic toxicity study; **CANNOT BE UPGRADED**-only 76 weeks of exposure (continued unexposed for up to 36 weeks), dose levels not high enough (failure to demonstrate an MTD), only two dose levels, no ophthalmoscopy, inadequate serum chemistry, inadequate histopathology, and the total number of animals examined for tumors is fewer than the total initially on study. Luthra 12/29/87, Davis 7/11/88.

50395-032 124668 Duplicate of 017 055701, above.

011 043236, 043237 "Review of Recent Toxicology Studies on p-Dichlorobenzene" Fd. Chem. Toxic. 21:825-832, 1983. Includes a summary of the combined inhalation study in rats. Davis 8/14/86.

011 043234 A brief summary of the combined inhalation study in rats. Davis 8/14/86.

009 036703 Very brief summary of the publication in Record 043237 although the journal name is stated incorrectly. Remsen 1/17/86.

003 003855 (1956, Publication) Very brief summary; liver and kidney effects. Remsen 4/2/85.

50395-032 124669 Exact duplicate of 003:003855, above.

CHRONIC, MOUSE

022 064739 "Paradichlorobenzene: Long Term Inhalation Study in the Mouse." (Imperial Chemical Industries Limited, 7/10/80). Paradichlorobenzene (99.8% pure) was administered in the atmosphere to Alderley Park Swiss mice at 0 (air), 75 and 500 ppm (25/sex/group) for 57-61 weeks (males) or 57 weeks exposure to p-DCB and no treatment until termination at 75-76 weeks (females). NOEL > 500 ppm (no significant effects were observed at any level). **Not acceptable** (there was a suspected Sendai virus infection during the study which apparently induced severe lung and respiratory passage pathologies; housing was such that mortality was increased in males by excessive fighting; three dose levels should have been tested instead of only 2; body weight and food consumption were not measured; males only were tested for hematology, clinical chemistry and urinalysis and all the required parameters were not tested; an eye exam was not performed; organ weights for liver, kidneys, brain and testes were not taken). **Not upgradeable** (an MTD was not reached but data could not be reasonably interpreted when the animals had a lung virus infection; the report is missing too much information). M. Silva, 8/29/88.

011 043238, 043236, 043235 "Review of Recent Toxicology Studies on p-Dichlorobenzene" (1983, Institute of Toxicology, Bayer AG and Central Toxicology Laboratory, Imperial Chemical Industries plc.) Paradichlorobenzene, 99.8% purity. Record # 043238 describes a 57-week inhalation test done on 75/sex/group SPF Swiss mice (Alderly Park strain) at dose levels of 0, 75 and 500 ppm. Animals were group-housed (9-10/cage) and subsequent fighting as well as probable respiratory infection reduced the male population to 20% of its original numbers by termination of the dosing. Consequently, the study was continued only on the females. A tumor incidence table is included. Record # 043236 is the abstract for the summarized study in 043238. Record # 043235 is a short summary of 043238. **NO ADVERSE EFFECT; UNACCEPTABLE, INCOMPLETE**. Important deviations from the guidelines include the 57 week exposure period; the two dose levels; the lack of differential blood counts and ophthalmology; the respiratory disease in both sexes; and the mortality rate for males. Because this is published material there is too little detail to fully evaluate the protocol or results. Davis 8/14/86. [This appears to be a review of 022:064739, above. C. Aldous, 1/13/89.]

009 036704 Very brief summary of the publication in Record 043238, although the journal name is stated incorrectly. Reviewed by Remsen, 1/17/86.

CHRONIC TOXICITY, DOG (INCLUDES SUPPORTIVE SHORT DURATION STUDIES)

50395-043 145808 Naylor, M.W. and L.D. Stout, "One year study of *p*-dichlorobenzene administered orally via capsule to beagle dogs", Ceregen (A Unit of Monsanto Company EHL), 3/25/96. Laboratory Project No. EHL 94093. Five beagle dogs/sex/group were administered 0, 10, 50, or 75 mg/kg/day *p*-dichlorobenzene (99.9%) by gelatin capsule for 1 year. [The high dose groups had begun the study at 150 mg/kg/day, but excessive toxicity (including deaths or premature sacrifices of 3 high dose dogs) necessitated a 2-wk recovery period, with establishment of 75 mg/kg/day as the high dose level from the sixth week onward]. No NOEL was identified, however it could be extrapolated to be only slightly below 10 mg/kg/day. [Effects at 50 mg/kg/day included hepatocellular changes, particularly hypertrophy and pigment deposition; corresponding marked increases in serum alkaline phosphatase and ALT, and decreased serum albumin; and liver weight increases. Multifocal hepatocellular hypertrophy in one 10 mg/kg/day female was also considered to be treatment-related]. Modest increases in adrenal and thyroid weights (without associated histopathology) were also seen at 50 mg/kg/day and above. Small decrements in RBC counts and HCT and associated increases in platelet counts were seen at 50 to 75 mg/kg/day. This study is **acceptable, and there are no adverse effects indicated. Since this study provides a valid characterization of toxicity in a comparatively sensitive species, **there is also no need for a repeat rat chronic study at this time**. Aldous, 4/11/96.

50395-038 132561 This is a letter to DPR regarding mortalities in the dog chronic study (50395-043 145808), above.

50395-035 130670 Draft protocol for a 6-month dog study. The study design was modified to 1 yr, and results are reported as Record No. 145808, above. Comments on the draft protocol were made by Aldous, 6/30/94.

50395-040 134441 Harrington, R.M. and D.C. Thake, "Four week range-finding study of para-dichlorobenzene administered by capsule to beagle dogs", Laboratory Project No. EHL 94058. Investigators evaluated 2/sex/group at 25, 75, 150, and 300 mg/kg/day. Both high dose males died, at least one of them attributable to test article (the other died of a perforated esophagus, which may have resulted from a lodged capsule). Both 150 mg/kg/day males had markedly elevated circulating bilirubin levels, as well as elevated ALT, AST, and alkaline phosphatase. All groups of 75 mg/kg/day and above had increased liver weights, and several individuals at 75 mg/kg/day and above had evidence of marked gastrointestinal tract irritation. The latter was often observed as discoloration of the duodenum or jejunum. The low dose of 25 mg/kg/day appeared to be a NOEL. The dose levels selected for the chronic study were appropriately selected, based on this 4-wk study. (No DPR worksheet: this information was noted in the review of chronic dog study, 50395-043 145808).

50395-035 130671 Lumley, C.E., Parkinson, C., and Walker, S.R., "An international appraisal of the minimum duration of chronic animal studies", Human & Experimental Toxicology 11, 155-162 (1992). Noting that some countries require 6 months for chronic studies in support of new drugs, whereas others require 12 months for chronic studies, investigators evaluated outcomes of studies in cases in which both 6-month and 12-month studies were available for

pharmaceuticals. They noted that new chronic findings were obtained after 12 months in 9/75 cases for which comparable 6-month studies were available. In none of these instances did the differing outcomes affect whether further development of respective drugs should continue. In summarized case-by-case comparisons, investigators noted that in many cases the new findings obtained in 1-yr studies would have been expected based upon findings in the 6-month studies, or upon the basis of known pharmacologic effects. This report was submitted to support a request to perform a 6-month dog study rather than a 1-yr dog chronic study for para-dichlorobenzene. No DPR worksheet is appropriate for this article. Aldous, 6/30/94.

50395-027 121096 "A Four Week Inhalation Toxicity Study of Paradichlorobenzene in the Dog", (Paul E. Newton, Bio/dynamics, Inc., East Millstone, N.J., Report # 88-8148, 22 February 1991). Paradichlorobenzene (100% a.i.) was used in inhalation chambers [whole-body exposures] for 6 hr/day, 5 days/wk, for 4 wk at estimated levels of 0, 58, 168, and 329 ppm [the latter dose estimate is inexact due to technical problems]. There were 2 beagles/sex/group. Group mean food consumption and body weights were reduced for both sexes at the high dose level. One high dose male died on day 19: this was considered to be a treatment effect. Increased terminal levels of SGOT, SGPT, alkaline phosphatase, triglycerides, and gamma glutamyl transpeptidase were recorded for both sexes at the high dose. Increased relative liver, adrenal, and kidney weights and reduced spleen weights were found for both sexes at the high dose. Necropsy revealed enlarged adrenals (both sexes) and lung surface irregularities (females) at the high dose. Microscopy indicated hepatic lipidosis in both sexes at the high dose. No other microscopic findings were of sufficient frequency and degree to be considered definite treatment effects. **No adverse effects indicated**. Apparent NOEL = 168 ppm. Acceptability is not an issue, since this is a range-finding study. (H. Green and C. Aldous, 4/7/93).

ONCOGENICITY, RAT

**** 022 064719** "Toxicology and carcinogenesis studies of 1,4-dichlorobenzene (CAS No. 106-46-7) in F344/N rats and B6C3F1 mice (gavage studies)" NTP Technical Report No. 319, performed at Battelle Columbus Laboratories, January, 1987. Dosages of 0, 150, and 300 mg/kg/day in male rats: 0, 300, and 600 mg/kg/day in female rats. Treatment 5 times/week by gavage in corn oil. No NOEL was sought nor found in this study. Kidney was the primary target organ: at the lowest dose tested (LDT) of 150 mg/kg/day in males, there was increased degree of nephropathy, increased mineralization in collecting tubules of the medulla, increased focal hyperplasia in the tubules, and increased epithelial hyperplasia in the pelvis. Parathyroid hyperplasia was observed in all treated males, and may have been a response to kidney toxicity. Females also had increased incidence of neuropathy at the LDT of 300 mg/kg/day, however kidney effects were less severe than in males. Tubular cell adenocarcinomas were increased in males (incidence of 1/42, 3/42, and 7/46 in controls through increasing dosage groups). Findings, particularly the kidney tubular cell adenocarcinomas, constitute "**possible adverse effects**". **Acceptable** as an oncogenicity study. [Note that a brief CDFA review of 8/19/88 classified study as unacceptable (summary data only), apparently based on lack of individual data for non-neoplastic lesions. This decision has been reversed in the 1/5/89 review, which considered data adequate to evaluate oncogenicity potential]. M. Silva, 8/19/88, C. Aldous, 1/5/89.

NOTE: The ancillary studies and interpreted material which follow in this section provide evidence that the kidney tumors and associated toxicity in male rat kidneys are part of a

syndrome unique to male rats, and well studied with respect to other nephrotoxins such as 2,2,4-trimethylpentane. This additional information should be considered in subsequent risk assessment. The case for p-dichlorobenzene effects being unique to male rats as presented in 022:064737, below, would be stronger if that report were complete. Additional "1-liners" of records in the same volume are found at the end of this "Summary of Toxicology Data".

022 064735 "1,4-Dichlorobenzene-Induced Nephrotoxicity: Similarity With Unleaded Gasoline (UG)-Induced Renal Effects," from: Nephrotoxicity: Extrapolation From in vitro to in vivo and From Animals to Man; Eds. P. Bach & E.A. Loch, In Press, Third International Symposium on Nephrotoxicity held in Guildford, England, 8/3/87 to 8/7/87. Studies in F-344 rats demonstrated similarities between 1,4-dichlorobenzene (1,4-DCB) and 2,2,4-trimethylpentane (TMP), including enhanced renal proximal tubule cell protein droplet formation and enhanced renal cell proliferation. Also, substantial amounts of 1,4-DCB and/or metabolites and TMP co-elute with the protein, a2μ-globulin, which is characteristic of male rat kidneys and which accumulates in TMP renal toxicity. The reversible association of 1,4-DCB to renal cytosolic proteins resembles association of TMP to a2μ-globulin. In contrast, 1,2-DCB (which, unlike 1,4-DCB and TMP, apparently does not elicit male rat renal toxicity and renal tumors) was less potent at droplet formation, did not cause renal cellular proliferation, and did not have the characteristic reversible association with proteins comparable to 1,4-DCB and TMP. Report was reviewed by M. Silva, and subsequently re-examined by C. Aldous to provide a statement about the relevance of this study to the NTP rat oncogenicity study (022:064719). Conclusion by C. Aldous was that "These data are consistent with, but do not demonstrate unequivocally, the thesis that 1,4-DCB and/or its metabolic product(s) have a reversible affiliation with a2μ-globulin, as does TMP." M. Silva, 8/25/88; C. Aldous, 1/12/89.

022 064737 "p-Dichlorobenzene: Subchronic toxicological studies on the subject of nephrotoxic effects in Fischer 344-rats (13-week trial with administration by stomach tube)". Bayer AG Institute of Toxicology, draft document, hence no completion date. Final autopsy completed 11/5/85. Report indicates a pattern of renal toxicity like that of 2,2,4-trimethylpentane (TMP), which is specific to male rats (see 1/13/89 review for additional evaluation of kidney effects). Liver hypertrophy was also noted in both sexes, dose related, at all dosages. Report is by design **Supplementary Data**: it is **highly recommended that the final draft be submitted**, and histopathology tables should be presented in those data. Note: original review indicated "possible adverse effects". This report is now re-classified as supplementary data, since it was designed to evaluate known chronic effects. M. Silva, 8/26/88; C. Aldous, 1/13/89.
015 50542 "White Paper on para-Dichlorobenzene" (Chlorobenzene Producers Association, 6/19/86) Position paper by the Chlorobenzene Producers Association on preliminary findings of a National Toxicology Program oncogenicity study in rats and mice. Liver tumors in mice of both sexes (600 mg/kg/day) and kidney tumors in male rats (150 and 300 mg/kg/day) were reported. The Association discounts the results as not applicable to human health because of the use of gavage, the negative results in a previous study which used inhalation, the negative results in mutagenicity assays, the susceptibility of the mouse strain to liver tumors, and the implication of a kidney protein in rats which is not found in humans. Davis 10/21/87. One-liner only.

019 060902 Exact duplicate of 064719 in 022.

003 003853 (No date given, Publication) Very brief summary; no adverse effect reported. Remsen, 4/2/85.

ONCOGENICITY, MOUSE

**** 022 064719** "Toxicology and carcinogenesis studies of 1,4-dichlorobenzene (CAS No. 106-46-7) in F344/N rats and B6C3F1 mice (gavage studies)". NTP Technical Report No. 319, performed at Battelle Columbus Laboratories, January, 1987. Dosages of 0, 300, and 600 mg/kg/day, 5 days/week, for 103 weeks by gavage in corn oil to 50 mice/sex/group. **Possible adverse effect**: increased hepatocellular adenomas and carcinomas in males and females at 600 mg/kg/day, also hepatoblastomas (rare tumor type) in males at 600 mg/kg/day. No other treatment-related tumors, but some evidences of toxicity at 300 mg/kg/day upwards included hepatocellular degeneration, necrosis, cellular alterations (both sexes, more prominent in males); hyperplasia in adrenal capsule (males); mandibular lymphoid hyperplasia (both sexes); and nephropathy (males). No NOEL was sought nor found in this study. **Acceptable as an oncogenicity study**. [Note that a brief CDFA review of 8/19/88 classified study as summary data only, apparently based on lack of individual data for non-neoplastic lesions. This decision has been reversed in the 1/17/89 review, which considered data adequate to evaluate oncogenicity potential]. M. Silva, 8/19/88, C. Aldous, 1/17/89.

See also 015:050542 under ONCOGENICITY, RAT.

NOTE: Volume 022, pp. 12-15 (prior to first tab) discusses the high spontaneous tumor rate of B6C3F1 mice, the possibility of an oncogene being the cause of such tumors, and indications that paradichlorobenzene may increase the mouse tumor rate over historical background incidence by a non-genotoxic mechanism, as appears to be the case for several other chlorinated hydrocarbons. Reviewer (C. Aldous) notes in addition to these comments, that there was marked toxicity to livers of males and females at both doses tested, thus increased tumor incidence could indicate an enhancement of background incidence in association with severe liver toxicity. Human risk assessment should be performed in context of these observations (C. Aldous, 1/17/89).

50395-045 152815 (no named author) Translation of a summary data table from a Japanese mouse oncogenicity study. Crj:BDF1 mice and Fischer rats were apparently tested, however this record addressed only mouse results. Submitted data consist of one small table obtained by the Chlorobenzene Producers Association. The table indicated increases in hepatocellular tumors in both sexes, histiocytic sarcomas in males, and alveolar/bronchiolar carcinomas in females at 300 ppm. There were no reported tumor effects at 75 ppm or lower dose levels. The study was not performed by or for the submitters, and they have no basis for verifying validity of the data. No worksheet (no reviewable data). Aldous, 3/5/97.

REPRODUCTION, RAT

** **023 73131** "Two-Generation Reproduction Study of Inhaled Paradichlorobenzene in Sprague-Dawley (CD) Rats", (Chlorobenzene Producers Association, 1/16/89). Paradichlorobenzene, 99.99% pure, was administered via inhalation to 28 Sprague-Dawley rats/sex/group (with an additional 10 females/exposure level) at target concentrations of 0, 50, 150 or 450 ppm (actual concentrations 0, 66.3, 211, and 538 ppm), 6 hours a day, 7 days a week, 10 weeks prior to, and during mating. Pregnant females continued on this schedule through day 20, started again on postpartum day 5, and continued through lactational day 27. **Possible adverse effects:** Developmental Toxicity (pre- and postnatal growth deficiency as measured by pup weight, increased rate of perinatal death, and decreased litter size). Parental Toxicity (tremors, decreased body weight, renal and hepatic hypertrophy with associated histological changes). Developmental NOEL = 150 ppm (increased perinatal death, decreased litter size, decreased pup weight); Maternal NOEL = 50 ppm (increased liver weight); Paternal NOEL < 50 ppm (hyaline droplet nephropathy). **Acceptable.** (D. Shimer and G. Chernoff, 8/2/89)

NOTE: The U.S. EPA "Core" classification for this study was "Supplementary" as of 1990, due to inadequate analysis of test atmosphere and to lack of a NOEL for males.

TERATOLOGY, RAT

** 50395-026 120265 Neeper-Bradley, T.L. and Kubena, M.F., "Developmental toxicity study of maternally inhaled paradichlorobenzene (PDCB) vapor in CD* rats", Bushy Run Research Center (BRRC), 12/23/92. Dams were mated at about 10 weeks, then exposed to paradichlorobenzene (99.0% purity, Lot No. 11 07 91 DGB) by inhalation on gd 6-15 for 6 hr/day, at target doses of 0, 50, 200, and 600 ppm [mean analytical chamber atmosphere concentrations were 49, 203, and 615 ppm]. Maternal NOEL = 50 ppm (modest b.w. and food intake decrements, blepharospasm [eyelids partially closed due to tonic muscle contraction] during exposure sessions, perioral wetness in between exposures). Developmental NOEL = 200 ppm (delayed ossification of cervical bones). **Acceptable, with no adverse effects**. Aldous, 4/6/93.

017 055702 Final report of record # 043239. "Para-Dichlorobenzene: Teratogenicity Study in Rats" (ICI Central Toxicol. Lab., Cheshire, UK. Report # CTL/P/340, 7/22/77) Specific Pathogen Free mated rats (Alderly Park), 32/group, were exposed to 0, 75, 200 and 500 ppm of p-DCB by inhalation for 6 hrs/day, daily on days 6-15 of gestation. Pups were delivered by C-section from at least 20 dams/group on day 20 of gestation. **NO ADVERSE EFFECT**-No developmental or maternal effects reported. Developmental and maternal NOEL = 500 ppm (HDT). **UNACCEPTABLE**: No rationale for dose selection; overt maternal toxicity (MTD) not elicited at 500 ppm; no historical control data; necropsy, histopathology and clinical observation

data incomplete; Potentially upgradeable if dose selection can be justified and additional data are available. Y. Luthra 1/7/88.

011 043239 "Para-dichlorobenzene: Teratogenicity Study in Rats." (1983, Central Toxicology Laboratory, Imperial Chemical Industries, plc.) Condensed overview of the final report # 055702.

017 055703 "Teratologic Evaluation of p-Dichlorobenzene in the Rat." Bull. Environ. Contam. Toxicol. (1986) 37:164-168 (University of Milan, Italy). p-DCB, 99% purity, given by gavage (2.5 ml/kg) to mated CD rats (sperm detection = day 1) on gestation days 6-15 at 0 (corn oil), 250, 500, 750 and 1000 mg/kg. **NO ADVERSE EFFECTS** reported. Maternal NOEL = 250 mg/kg, reduced weight gain & food consumption; developmental NOEL = 250 mg/kg, dose related increase in frequency of extra ribs at 500 mg/kg and higher doses, reduced fetal weight at 1000 mg/kg. **UNACCEPTABLE**: Journal article lacking sufficient detail for independent review. Too few pregnant rats/dose/group. No dosing solution analysis; dam and pup individual data, and historical control data not included; no summary data for necropsy/clinical observations; GLP/QA statement omitted. Not upgradeable. Y. Luthra 1/12/88.

009 036701 (No date or lab given) Very brief summary. Remsen, 1/17/86.

011 043245 (No date or lab given) Half page summary; same study as in 009 036701.

TERATOLOGY, RABBIT

** 009, 011, 016 036693 "Paradichlorobenzene: Inhalation Teratology Study in Rabbits." (Toxicology Research Laboratory, Dow Chemicals, 9/15/82). Paradichlorobenzene, 99.9% purity, at chamber concentrations of 0, 100, 300, and 800 ppm to 30 New Zealand White rabbits/group for 6 hours/day, gestation days 6 through 18, by inhalation. **NO ADVERSE EFFECT**. Maternal NOEL = 300 ppm (decreased body weight gain), developmental NOEL = 800 ppm. **ACCEPTABLE** - initial review (Parker 1/21/86 found the study unacceptable); individual doe and fetal data in record # 054969 correct the deficiencies. J. Parker, 1/21/86, B. Davis, 7/16/87, Luthra 1/8/88.

009 046242 "Paradichlorobenzene: Inhalation Teratology Probe Study in Rabbits," (Dow Chemical, 2/16/82). Paradichlorobenzene (99.9% pure) was administered by inhalation (6 hours/day) to inseminated New Zealand White rabbits (7/group) at 0, 300, 600, or 1000 ppm during gestation days 6-18 (day 0 = day of insemination). **No adverse effect. The 1000 ppm group had slightly reduced body weight gain and modest liver alterations, and the high dose selected for the definitive study was 800 ppm.** The primary teratology study (Record #036693) was previously reviewed as unacceptable (J. Parker, 1/21/86, D. Shimer/ B. Davis, 7/16/87). The 7/16/87 review was ambiguous as to whether pilot or primary study was being addressed and appeared to be requesting individual data on the pilot study in addition to historical data of value in interpretation of the primary study. The information apparently requested from the pilot study was submitted (Record #064788) and a CDFA review of 8/22/88 classified the pilot study as "acceptable". Since the primary study had also been accepted by CDFA on 1/8/88, the meaningfulness of an "acceptable" status for a pilot is a moot issue. J. Parker, 1/21/86, B. Davis, 7/16/87, M. Silva, 8/22/88, C. Aldous, 1/17/89 (one-liner update only).

017 054969 Addendum to Record # 036693. Contains data and information on individual does and fetuses making the report complete. D. Shimer/Y. Luthra 1/8/88.

009 036702 (1982, Dow Chemical) Very brief summary of study in Record 036693. Parker 1/21/86.

009 57052 A 2-page commentary (addendum) to Record #036693, above.

GENE MUTATION

017 055808 "Mutagenicity Evaluation of p-Dichlorobenzene in the CHO HGPRT Forward Mutation Assay" (Litton Bionetics, Netherlands, 5/86, Report # 3691.) P-Dichlorobenzene (batch # 922, >99.9% purity) was assayed for mutagenic activity at the HGPRT locus in CHO cells from 80-240 μg/ml without activation and from 70-350 μg/ml with activation. **NO ADVERSE EFFECT**-possible mutagenicity with activation was not confirmed in the repeat experiment. **UNACCEPTABLE -** No confirmatory assay was done in the absence of activation. Davis 7/8/88.

017 055804 Full study report for 011 043240, 043250. "Paradichlorobenzene: Estimation of its Mutagenic Potential in the Salmonella typhimurium Plate Incorporation Mutagenicity Assay" (ICI, Central Toxicology Lab., Nov 1976, Report No. CTL/P/298) Paradichlorobenzene, purity unspecified, was tested in the Ames assay on strains TA1535, TA1538, TA98, and TA100, with and without activation (S9 from rat liver, pretreated with Aroclor) in gaseous phase at concentrations of 94, 299, or 682 ppm (Experiment 1), dissolved in DMSO at 100, 500, or 2500 ppm (Experiment 2), dissolved in DMSO at 4, 20, 100, 500, or 2500 ppm (Experiment 3). **NO ADVERSE EFFECT**-negative for mutagenesis; **UNACCEPTABLE** - serious design flaws, pooling of heterogeneous data, no individual plate data, inadequate positive controls, no test material characterization, uncertain exposure times. Davis 7/7/88.

011 043240, 043250 "Review of Recent Toxicology Studies on Paradichlorobenzene: Mutagenicity" and "Paradichlorobenzene (PDCB): Estimation of Its Mutagenic Potential in the Salmonella typhimurium Plate Incorporation Mutagenicity Assay" (1983, Central Toxicology Laboratory, Imperial Chemical Industries, plc.) Both of these submissions are short summaries of Record 055804. Davis 8/14/86.

** 013, 022 046241, 064789 "Final Report on Salmonella Mutagenicity Assay of p-Dichlorobenzene," (Monsanto Chemical Company, 7/78). P-Dichlorobenzene technical (purity > 99.5%) was tested in both spot test and plate incorporation assay with Salmonella strains TA98, TA100, TA1535 & TA1537 with and without metabolic activation at 0, 0.6, 2.4, 12, 60, 180 or 600 μg/plate (triplicate plates). **No increase in mutation frequency was observed at any dose.** The study was originally reviewed as unacceptable (D. Shimer/B. Davis, 7/16/87) however the information requested by CDFA was submitted for evaluation (protocol, missing and duplicated pages & discussion of specific issues brought up in the report). The submitted information (022 064789) was evaluated and the study is now complete and **acceptable**. M. Silva, 8/22/88.

011 043252 "In vitro Microbiological Genotoxicity Assays of Chlorobenzene, m-Dichlorobenzene, o-Dichlorobenzene, and p-Dichlorobenzene. Final Report." (5/79, SRI

International) Paradichlorobenzene, no purity given. Standard Ames assay on Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 plus Escherichia coli strain WP2 reverse mutation assay. One test with 50, 100, 250, 500, 750, and 1000 μg/plate and repeat test with 0.5, 1.0, 10.0, 50.0, 100, and 500 μg/plate. **NO ADVERSE EFFECT INDICATED; UNACCEPTABLE**-Insufficient information on test substance, protocols, or Good Laboratory Practice compliance, inadequate dose levels with activation. Davis 8/20/86.

009 036694 (1977, no lab given) Summary - Ames assay. Remsen, 1/17/86.

009 036695 Very brief summary of 009 036694. Remsen, 1/17/86.

011 043255 "Effect of PDCB on Genetic Material - Host Mediated Assay" (6/5/75, Huntingdon Research Centre) Paradichlorobenzene, purity not stated. Male CFLP mice were dosed by oral gavage with 0, 4, 8, or 16 g/kg bodyweight of paradichlorobenzene and then inoculated i.p. with a suspension of Salmonella typhimurium strain G46. After 2.5 hours, the bacteria were harvested and plated on both histidine deficient and histidine rich media to assay for reverse mutations and for total colonies. **NO ADVERSE EFFECT INDICATED; INCOMPLETE, UNACCEPTABLE**. Not an acceptable assay system for mutagenicity. Davis 8/20/86.

022 064719 "Toxicology and carcinogenesis studies of 1,4-dichlorobenzene (CAS No. 106-46-7) in F344/N rats and B6C3F1 mice (gavage studies." NTP Technical Report No. 319, Battelle Columbus Laboratories, 1/87) Technical 1,4-dichlorobenzene (>99%) tested with Salmonella strains TA1535, TA1537, TA98 and TA100 with and without Aroclor induced rat and hamster liver activation at 0 (DMSO), 1, 3.3, 10, 33 and 100 mg/plate in triplicate, two trials; 48 hour incubation; results of one trial (mean ± standard error) reported; no evidence of increase in reversion rate and no clear evidence of cytotoxicity to justify concentrations used; **unacceptable** (no protocol, single page of data only, no cytotoxicity). **No adverse effect.** No worksheet. J. Gee, 1/20/89.

011 043247 "Drosophila Sex Linked Recessive Lethal Test on Para-dichlorobenzene" (University of Wisconsin, Madison, 10/82) Para-dichlorobenzene, no purity stated. Parental wild type Drosophila males were exposed by static inhalation to 0, 6000, or 15,600 ppm x hr in a series of three experiments. They were then mated sequentially to three sets of Basc virgin females to assay all post-meiotic stages of male gametes. F1 daughters were mated individually and their offspring scored for the presence of sex-linked recessive lethals. **NO ADVERSE EFFECT INDICATED; UNACCEPTABLE, INCOMPLETE**. This is a draft report and as such the authors indicate that it is incomplete. Further, the broods were only intended to assay post-meiotic stages, though the guidelines require earlier stages as well. Davis 8/15/86.

009 036697 (No date or lab given) Very brief summary of sex-linked recessive lethal assay. Remsen, 1/17/86.

022 064719 "Toxicology and carcinogenesis studies of 1,4-dichlorobenzene (CAS No. 106-46-7) in F344/N rats and B6C3F1 mice (gavage studies." NTP Technical Report No. 319, Battelle Columbus Laboratories, 1/87) Technical 1,4-dichlorobenzene (>99%) tested with mouse lymphoma L5178Y/TK+/- cells with and without activation - activation with S9 prepared from Aroclor-induced livers of male F344 rats; treated for 4 hours without activation at 0 (1% DMSO), 65, 85 or 95 mg/ml in duplicate, two trials; with activation, 4 hours treatment, three trials at 0 (1% DMSO), 70, 80, 90 or 100 mg/ml, trial A, at 0, 65, 85 or 95 mg/ml, trial B and at 0,

80, 85, 90, 95 or 100 mg/ml, trial C with duplicates in each trial; triflurothymidine to select; 15 mg/ml MMS without activation and 2.5 mg/ml 3-methylcholanthrene with activation as positive controls; tested to adequate cytotoxicity; no increase in mutation frequency in a concentration-dependent manner; **no adverse effect; unacceptable** (inadequate report with 2 pages of data and minimal description of the conduct of the test as footnotes only). No worksheet. J. Gee, 1/20/89.

CHROMOSOME ABERRATIONS

Overview: The data gap is filled by the acceptable study, Record 055807. The results of the studies in this category are, with one exception, negative. Although mutagenesis was reported in the Aspergillus assay, this cannot be evaluated since the submission was a brief summary. Therefore, there is no compelling evidence for an adverse effect. [Comment by Davis (11/02/87 revision), updated by Silva (9/6/88 revision)].

** 017 055807 "Investigation of p-Dichlorobenzene for Clastogenic Effects in Mice Using the Micronucleus Test" (Bayer AG Institute of Toxicology, Report # 14694, 6/10/86) Bor:NMRI strain of mice, 5/sex/group, given 2500 mg/kg paradichlorobenzene (99.9%) by gavage, 10 ml/kg in maize oil. Bone marrow samples were processed 24, 48, and 72 hours post-treatment. Cyclophosphamide, 20 mg/kg p.o., as positive control; samples processed at 24hours. Micronuclei/1000 PCE/animal counted. **NO ADVERSE CLASTOGENIC EFFECTS**. NOEL >2500 mg/kg. Report **COMPLETE** and **ACCEPTABLE.** Luthra 1/22/88.

017 055806 "Paradichlorobenzene: Cytogenetic Study in the Rat." (ICI Report # CTL/P/293, 11/76) Male rats were exposed to Paradichlorobenzene, purity unspecified, by inhalation; (Expt. 1) 3/group to a single 2 hour exposure at 299 or 682 ppm; (Expt. 2) 2/group at 75 or 500 ppm for 5 hours/day for 5 days; (Expt. 3) 2/group at 75 and 500 ppm for 5 hours/day, 5 days/week for 3 months. Animals were killed 22 hrs after exposure and 50 or 100 bone marrow cells per animal were investigated. **NO ADVERSE EFFECT-**no chromosome mutagenicity induced by paradichlorobenzene. **UNACCEPTABLE**, **CANNOT BE UPGRADED -** Use of only male rats, inadequate numbers of animals, inadequate numbers of dose levels, inadequate justification for dose level selection, no test material characterization, ineffective positive controls, only one sacrifice interval, no data for actual chamber concentration. Luthra 1/22/88, Davis 7/14/88.

011 043242 "Review of Recent Toxicology Studies on Paradichlorobenzene: Mutagenicity, In Vivo Systems - Cytogenetic Assay on the Bone-Marrow Cells of Rats." (1983, Central Toxicology Laboratory, Imperial Chemical Industries, plc.) Summary of the results of the full chromosome aberration assay in Record 55806. Davis 8/14/86.

011 043248 "Effects of Paradichlorobenzene on the in vitro Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells." (6/22/82, Bioassay Systems Corporation) Paradichlorobenzene, 99.7% purity. Chinese hamster ovary (CHO) cells were treated with 0, 50, 30, 20, 10, or 5 μg/ml test material, with and without activation. The 10 and 5 μg/ml groups were not analyzed. The other groups were analyzed for chromosome aberrations. Duplicate cultures were done for each dose level in each of two separate experiments. **NO ADVERSE EFFECT INDICATED; INCOMPLETE, UNACCEPTABLE**. Correctable deficiencies: no test material information, no cytotoxicity information for chromosome aberration assays, no cell

culture methods or contamination tests, missing data, insufficient data analysis. Noncorrectable deficiencies: range finding tests inadequate, dose levels inappropriate, only one harvest, multiple problems with negative control results. Davis 8/18/86.

011 043249 "Effects of Paradichlorobenzene on the in vitro Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells." (1/12/83, Bioassays Systems Corporation) Paradichlorobenzene, 99.7% purity. Chinese hamster ovary (CHO) cells were treated for 12 hours with 0, 50, 100, or 150 μg/ml in one nonactivation assay (assay A35) and with 0, 150, or 200 μg/ml in a second nonactivation assay (assay A37), or for two hours with 0, 50, 100, 150, or 200 μg/ml in an activation assay (assay A38). Results from earlier assays (assays A9, A22, A14, A25) are included, but with unexplained changes in the data and analysis (see Record #043248). **NO ADVERSE EFFECT INDICATED; INCOMPLETE, UNACCEPTABLE**. Deficiencies: missing test material information, inconsistencies with previous report (Record #043248), inconsistency in cytotoxicity results, no cytotoxicity information for chromosome aberration assays, numerous deviations from protocol, insufficient data analysis, missing data, range finding assay problems, negative control variability, cyclophosphamide treatment values. Davis 8/19/86.

017 055805; 011 043241 & 043246 "Paradichlorobenzene: Dominant Lethal Study in Mouse" (ICI, UK, 11/76) Sixteen male CD-1 mice/group were exposed to paradichlorobenzene at 75, 225 or 450 ppm for 6 hours/day for 5 days via inhalation. Thirty-five control male were exposed to air. Males were then mated with new virgin females each week for 8 weeks. Statistically significant observations were: 1) Increased number of females with one or more early deaths (225 ppm, week 1), 2) Increased early deaths as a percent of total implantations per pregnancy (225 ppm, week 6), 3) Decreased total implantations (75 and 450 ppm, week 8), 4) Decreased fertility (75 ppm, weeks 6, 7). The authors place no importance on these observations. The brief summaries of the study that were reviewed were found to be incomplete and unacceptable: insufficient data and information to evaluate the protocol and the results (Davis 8/15/86). Following the re-review of the additional data and information, the status remains unchanged as incomplete and **UNACCEPTABLE -** No data and/or information on chemical purity, inhalation chamber exposure concentrations, method of animal randomization and QA/QC audit. Table 3 is missing from the report. **NO ADVERSE EFFECTS INDICATED.** NOEL = 450 ppm. Upgradeable. D. Shimer/Y. Luthra 1/20/87.

009 046875 (1970, Publication) Very brief summary. Aspergillus nidulans showed some mutagenic activity. Remsen, 1/17/86.

009 036696 (No date or lab given) Very brief summary - cytogenetics assay. Remsen, 1/17/86.

011 043251 (No date or lab given) Very brief summary - same cytogenetic assay as in 009 036696.

009 036700 (1983, no lab given) Very brief summary - Dominant lethal mice. Remsen, 1/17/86.

022 064719 "Toxicology and carcinogenesis studies of 1,4-dichlorobenzene (CAS No. 106-46-7) in F344/N rats and B6C3F1 mice (gavage studies." NTP Technical Report No. 319, Battelle Columbus Laboratories, 1/87) Technical 1,4-dichlorobenzene (>99%) tested with

Chinese hamster ovary cells for sister chromatid exchange induction with and without activation with S9 from livers of male Sprague-Dawley rats induced with Aroclor 1254; incubated 2 hours in presence of paradichlorobenzene followed by an additional 22 - 26 hours with BrdU; mitomycin C and cyclophosphamide as positive controls; tested at 0 (DMS0, 1%), 75, 100, 125 or 150 mg/ml; **no adverse effect, unacceptable** (summary only). No worksheet. J. Gee, 1/20/89.

022 064719 "Toxicology and carcinogenesis studies of 1,4-dichlorobenzene (CAS No. 106-46-7) in F344/N rats and B6C3F1 mice (gavage studies." NTP Technical Report No. 319, Battelle Columbus Laboratories, 1/87) Technical 1,4-dichlorobenzene (>99%) tested with Chinese hamster ovary cells for chromosome aberrations with and without activation with S9 from male Aroclor-induced Sprague-Dawley rats; without activation at 0 (1% DMSO), 50, 100 or 150 mg/ml, mitomycin C (0.50 mg/ml) as positive control; with activation at 0, 25, 50 or 100 mg/ml with 25 mg/ml cyclophosphamide as positive control; inadequate information for evaluation - summary only; **unacceptable** (inadequate report). No worksheet. J. Gee, 1/20/89.

022 064719 "Toxicology and carcinogenesis studies of 1,4-dichlorobenzene (CAS No. 106-46-7) in F344/N rats and B6C3F1 mice (gavage studies." NTP Technical Report No. 319, Battelle Columbus Laboratories, 1/87) Technical 1,4-dichlorobenzene (>99%); blood smears from the 13-week studies were made and scored for micronuclei in erythrocytes in males and females; doses were 0 to 1800 mg/kg; **no adverse effect**; **unacceptable** (summary only). No worksheet. J. Gee, 1/20/89.

DNA DAMAGE

Overview: The positive results in the mitotic recombination study (011 043253) are not concentration-dependent and are difficult to interpret from the report as presented. The overall consensus is that there is no adverse effect, and no compelling evidence for DNA damage/repair. J. Gee, 1/20/89.

** 022 064727 "Evaluation of the Potential of p-Dichlorobenzene to Induce Unscheduled DNA Synthesis or DNA Replication in the in vivo-in vitro Rat Kidney DNA Repair Assay (TASK II)," (SRI International, 5/87). Paradichlorobenzene technical (99.8% pure) was administered by gavage to Fischer-344 rats (3-4/sex/group) at 0 (corn oil = vehicle), 300, 600 and 1000 mg/kg. Rats were sacrificed at 16 hours for UDS determination and at 96 hours for DNA replication (percent of cells in S-phase). No UDS effects were observed in either sex at any dose level. The positive control streptozocin functioned as expected. In males, there was an effect on DNA replication. At 1000 mg/kg there was a significant increase in DNA replication in male kidney cells. This effect was not observed in females. The positive control of mercuric chloride functioned as expected for males but not for females in the DNA replication test (at the same dose level), indicating a possible sex-specific action for mercuric chloride. Initially reviewed as a possible adverse effect on DNA replication (Silva, 8/23/88), but reconsideration finds this effect on percent of cells in S-phase supports the kidney of the rat as a target organ rather than reflecting DNA damage/repair per se. **Acceptable.** M. Silva, 8/23/88 and J. Gee, 1/20/89.

** 022 064726 "Examination of the Potential of p-Dichlorobenzene to Induce Unscheduled DNA Synthesis or DNA Replication in the in vivo-in vitro Mouse Hepatocyte DNA Repair Assay (TASK I)," (SRI International, 4/87). Paradichlorobenzene technical (99.5% pure) was

administered by gavage to B6C3F1 mice at 0 (vehicle = corn oil), 300, 600 and 1000 mg/kg. Mice examined for UDS were sacrificed at 16 hours post-treatment and mice examined for DNA replication were sacrificed 48 hours post-treatment (3/sex/group/time point at 0 & 300 mg/kg; at ≥ 600 mg/kg, 3-5/sex/group/time point). No increase in UDS was observed at any dose level. There was an increase in DNA replication, however, with a significant increase observed in males at 1000 mg/kg and an increase was observed in females at all dose levels. Initially reviewed as a possible adverse effect (Silva, 8/23/88), reconsideration finds this effect on percent of cells in S-phase supports the liver as a target organ in the mouse rather than reflecting DNA damage/repair per se. **Acceptable.** M. Silva, 8/23/88 and J. Gee, 1/19/89.

011 043253 "In vitro Microbiological Genotoxicity Assays of Chlorobenzene, m-Dichlorobenzene, o-Dichlorobenzene, and p-Dichlorobenzene, Final Report: Mitotic recombination in Saccharomyces cerevisiae." (5/79 SRI International) Paradichlorobenzene, purity not stated. Saccharomyces cerevisiae strain D3 exposed to multiple dose levels from 0.0005 to 5.0 w/v or v/v and assayed for frequencies of mitotic recombination at one of the adenine metabolism gene loci. **POSSIBLE ADVERSE EFFECT** indicated by numerous increased mitotic recombination frequencies, even though these were inconsistent and irreproducible. **INCOMPLETE, UNACCEPTABLE**. No information on test material or dosing solutions, no GLP information. Inconsistencies in cytotoxicity and mitotic recombination frequencies indicate a major problem. Davis 8/20/86.

011 043254 "In vitro Microbiological Genotoxicity Assays of Chlorobenzene, m-Dichlorobenzene, o-Dichlorobenzene, and p-Dichlorobenzene, Final Report: Differential Toxicity/Repair Assay in Escherichia coli and Bacillus subtilis" (5/79, SRI International) Paradichlorobenzene, purity not given. Paired strains of repair-competent and repair-deficient E. coli (W3110/p3478) and B. subtilis (H17/M45) were exposed to 1.0 or 5.0 mg of paradichlorobenzene for 16-17 hours in a disc diffusion growth inhibition assay. **NO ADVERSE EFFECT INDICATED. INCOMPLETE, UNACCEPTABLE**-No test article information, insufficient protocol, no evidence of GLP compliance, only two dose levels, no activation assays, no vehicle control, questionable positive control with E. coli in Experiment 1. Davis 8/20/86.

017 055809 "Evaluation of p-Dichlorobenzene in the In Vitro Transformation of BALB/3T3 Cells Assay" (Litton Bionetics, Netherlands, 6/86, Bayer Report # R 3710) p-Dichlorobenzene (batch 922, purity 99.9%) was tested in the cell transformation assay at 0, 60, 80, 100, 120, and 140 μg/ml with Balb/3T3 mouse cells. Exposure was for 72 hours. **NO ADVERSE EFFECT**-negative for transformation. **UNACCEPTABLE**; **CANNOT BE UPGRADED-**17/20 concurrent negative control flasks were contaminated, no historical control data, no metabolic activation. Luthra 1/29/88, Davis 7/15/88.

NEUROTOXICITY

There are no hen studies. One acute rat study and one subchronic rat study (both by inhalation) have been submitted, as follows:

**50395-036 131790 Li., A.A., Thake, D.C., and Kaempfe, T.A., "Acute neurotoxicity study of para-dichlorobenzene in Sprague-Dawley rats", Monsanto Company, Environmental Health Laboratory, St. Louis, MO, 7/27/94. EHL Project No. 93097. Para-dichlorobenzene, purity

>99.5%, was administered via vapor inhalation at concentrations of 0, 50, 200 or 600 ppm in a single four hour exposure to 10 Sprague-Dawley rats/sex/group. Study objectives included neurobehavioral FOB, motor activity assessment, and histopathology of neuronal tissues. NOEL = 200 ppm (transient statistically significant treatment effects in protocol parameters were decreased forelimb and hindlimb grip strength in the FOB, and decreased motor activity: both only at day 0 in 600 ppm males). In addition, 600 ppm males and females were noted to be hypoactive, lethargic, and unresponsive to external stimuli during inhalation exposure. There were no treatment effects in subsequent FOB, motor activity, or histopathology assessments. **Acceptable, with no adverse effects.** (Kishiyama and Aldous, 1/26/95).

50395-037 131791 Branch, D.K., Li, A.A., Thake, D.C., and Kaempfe, T.A., "Subchronic neurotoxicity study of para-dichlorobenzene in Sprague-Dawley rats", Monsanto Company, Environmental Health Laboratory, St. Louis, MO; Aug. 1, 1994. EHL Project No. 93098. Para-dichlorobenzene, purity >99.5%, was administered via vapor inhalation at concentrations of 0, 50, 200 or 600 ppm, 6 hr/exposure, 5 days/wk (except holidays and neurotoxicity testing days), for a total of 60 to 63 exposures over a 95-day study period to 10 Sprague-Dawley rats/sex/group. Study objectives included neurobehavioral FOB, motor activity assessment, and histopathology of neuronal tissues. FOB and motor activity were evaluated pretest and at weeks 4, 8, and 13 on all animals. Terminal histopathology was performed on 5/sex of controls and 600 ppm rats. NOEL for endpoints of the neurotoxicity testing = 600 ppm (highest dose level). No NOEL exists for transient effects observed during exposure, since hypoactivity (rats not responding to external stimuli) was observed on several occasions in 50 ppm rats. Hypoactivity and labored breathing were consistently observed in 200 ppm and 600 ppm rats during treatment. Perioral wetness was occasionally observed in high dose rats during treatment. **Acceptable, with no adverse effects. (Kishiyama and Aldous, 1/27/95).

50395-034 127940 Preliminary information related to Record No. 131791, above. Provisional review by Aldous, 3/3/94.

STUDIES FOCUSING ON MALE RAT KIDNEY EFFECTS

022 064730-32 Evidence was offered in these records (Tab F, Vol. 022) that administration of p-dichlorobenzene to male but not female rats results in reversible binding of the chemical or a metabolite of the chemical to the male rat-specific protein, a2μ-globulin, which leads to its accumulation in lysozymes of the P2 segment of the nephron as protein droplets. This lysosomal dysfunction leads to increased cell death, with a concomitant increase in cell proliferation. The syndrome occurs in male rats only. Dr. Swenberg has compared the amino acid sequence of the a2μ-globulin with nearly 1100 human proteins with no striking amino acid homology. In addition, human urine contains only small amounts of protein whereas male rat urine contains large amounts. Based on this information there is a question of relevance of a2μ-globulin nephropathy and its associated increase in renal neoplasia in humans. Three data tables support Swenberg's hypothesis about the specificity of a2μ-globulin nephropathy to male rats and its specific induction by p-dichlorobenzene. **This information is supplementary.** M. Silva, 8/24/88.

022 064733 "Mechanism of Petroleum-Induced Sex-Specific Protein Droplet Nephropathy and Renal Cell Proliferation in Fischer-344 Rats: Relevance to Humans," (CIIT-date of study not stated). A 21-day histoautoradiographic study was undertaken to assess the intra-renal

localization of unleaded gasoline (UG)-induced cell proliferation. Male Fischer-344 rats (3/group) were exposed to 0, 2, 20, 200 or 2000 ppm UG (6 hr/day, 5 days/week for 3 weeks). Osmotic pumps filled with 3H-thymidine (3H-TdR) were implanted subcutaneously on day 12 to assure a constant release of 3H-TdR during the last week of exposure. Animals were killed 1 day after exposure and the kidney cells were processed for autoradiographic examination. A study similar to the above was also run with 2, 2, 4-trimethylpentane (a nephrotoxic component of UG used as a model compound) at 0, 0.2, 0.5, 2.0, 5.0, 20.0 or 50.0 mg/kg TMP. TMP at 5, 10, 50 or 500 mg/kg was also administered in a single dose. A dose dependent increase in cell proliferation specifically in the proximal tubule segments with increased protein droplet formation was observed to be induced by UG and TMP after the 21 day treatment. Administration of a single dose of TMP increases protein droplet formation and DNA synthesis in kidney cells. The biochemical approach used to study the induction of protein droplet nephropathy has focused on the relationship between TMP and a low molecular weight protein produced only by the male rat, a2μ-globulin. Humans do not produce a2μ-globulin but similar low molecular weight proteins are reabsorbed in their kidneys. However, specific molecular characteristics of the a2μ-globulin appear to be necessary for the reversible binding of the TMP metabolite. Further studies are needed to understand the specificity of binding and properly assess the potential risk of hydrocarbon-induced protein droplet nephropathy in humans. **This information is supplementary.** M. Silva, 8/24/88.

022 064736 "Kidney-Specific DNA Repair Assay: An Evaluation of Unleaded Gasoline," (Published in CIIT Activities, Vol. 6, #4, 4/86). Unleaded gasoline has been found to cause kidney tumors in male rats. Kidney-specific short-term tests developed by CIIT's genetic toxicologists suggest that this agent, a mixture of hundreds of hydrocarbons, is not directly genotoxic in the kidney, but rather forces cell division by a toxic mechanism unique to the male rat. Intensive interdepartmental research at CIIT during the coming year will approach the mechanism of gasoline-induced kidney damage from several angles. **This information is supplementary.** M. Silva, 8/23/88.

022 064740 "Hydrocarbon-Mediated Nephrotoxicity," (From an article in the CIIT newsletter: CIIT Activities, Vol 5, #5 from May, 1985). This report contains a summary of the ongoing research on the mechanism of hydrocarbon-mediated nephrotoxicity, particularly as it relates to the appropriateness of the male rat as a surrogate model for humans. Evidence indicates that an alteration of the renal handling of a male rat-specific protein might be responsible for hydrocarbon-induced nephrotoxicity. The findings of the project will impact directly on the assessment of human risk resulting from the observation that chronic inhalation exposure to gasoline produces increased incidence of renal tumors in male rats. In particular, if the nephrotoxicity of gasoline can be linked to a toxicological event unique to the male rat it can be concluded that a similar event is not likely to occur in humans. In addition, a number of compounds of varied chemical structures have now been shown to produce male rat nephrotoxicity as a primary toxic endpoint. Thus these studies will enhance understanding of the usefulness of the male rat as a model for nephrotoxic effects of chemicals in humans. Finally, this project may also provide insight into how chemicals possessing no direct genotoxic activity ultimately can produce tumors in animal test systems. **This information is supplementary.** M. Silva, 8/25/88.

50395-033 126850 Bomhard, E., Luckhaus, G. and Voigt, W.-H. "p-dichlorobenzene: Subchronic toxicological study concerning the question of a nephrotoxic mechanism in Fisher 344 rats". Bayer AG, Institut fur Toxikologie, Wuppertal, 4/25/88. Ten

rats/sex/group were dosed daily by gavage with 0, 75, 150, 300, or 600 mg/kg/day of para-dichlorobenzene (99.9%). Five/sex/group were sacrificed after 5 wk, the others after 13 wk. Several parameters were examined, the most important of which was histological examination of kidneys. Only the summary page was translated into English. The utility of the report would be improved by translation of at least the pathology report and pathology tables, and by including a summary table of pathology findings (indicating "degrees" of pivotal findings). There was an expected sex difference in control kidneys, such that control males had small crystalline-like gram-positive structures in the tubular epithelia. Treated males had similar structures in various forms, often granular or spherical, found either within the tubular epithelium or in the lumina, and generally of higher "grade" of findings than were found in controls. These changes were evident at all dose levels tested. Tubular degeneration became evident at 150 mg/kg/day in some animals. Pathology became progressively greater at higher dose levels. Only tubules in or near to the cortical area were normally involved. Only the summary page was provided in English, however most parts of the report other than the histopathology had been provided in English as DPR Record No. 064737. The report would be improved by providing the histopathology data in English, and by including a summary histopathology table (preferably showing grades of lesions). Data are consistent with an a2μ-globulin mechanism, but do not prove the mechanism. Aldous, 12/23/93.

SUBCHRONIC OR SUBACUTE STUDIES WITH para-DICHLOROBENZENE

50395-032 124670 Auletta, C.S., "A 21-day dermal toxicity study in rats with para-dichlorobenzene". Bio/dynamics Inc. Project No. 88-3384, 10/26/89. CD* rats were dosed dermally with 0, 75, 150, or 300 mg/kg/day para-dichlorobenzene (100%) 5 days/wk for 3 wk. Study involved b.w. and food consumption measurements, hematology and clinical chemistry, gross necropsies and histopathology. Study summary stated that results were negative with a 300 mg/kg/day NOEL. No DPR worksheet is needed, as study does not address SB-950 data gaps. Aldous, 12/21/93.

50395-033 126848 Bomhard, E. and Luckhaus, G., "p-dichlorobenzene: Preliminary subacute toxicological study concerning the question of a hepatotoxic effect in mice", Bayer AG, Institut fur Toxikologie, Wuppertal, 9/15/86. NMRI mice, 8-10/group, were dosed with para-dichlorobenzene daily for 4 wk by gavage at 0 (corn oil vehicle), 300, 600, or 900 mg/kg/day. Week 4 clinical chemistry found dose-related elevated GPT at 600 to 900 mg/kg/day. Liver relative weights were elevated in dose-related fashion at the same dose levels. There was a marked, dose-related hypertrophy at 300 to 900 mg/kg/day in males and to a lesser extent at 600 to 900 mg/kg/day in females. Liver lipidosis was evident in both sexes, particularly evident in females at all dose levels. Investigators concluded that the above changes indicate increased liver metabolic enzyme induction, which may precede hepatocellular neoplasms, and hence that doses which are not hepatotoxic would not be expected to lead to increased liver neoplasms. Aldous, 12/22/93.

50395-033 126849 Bomhard, E. and Schmidt, U., "p-dichlorobenzene: Subacute toxicological study in Fisher 344 rats concerning the mechanism of enzymatic metabolism of foreign substances in the liver and kidney", Bayer AG, Institut fur Toxikologie, Wuppertal, Nov. 5, 1992 (English translation). Study was done mainly to evaluate enzyme induction in livers and kidneys of rats. Fischer 344 rats, 20/sex/group were dosed by gavage with 150 or 600 mg/kg/day of para-dichlorobenzene. Five/sex/group were killed 24 hr after dosing for either 2, 8, 14, or 28

days. Five controls/sex were killed after 2 or 28 daily vehicle (corn oil) treatments. Liver and kidney metabolic enzymes were assayed. The majority of liver metabolic enzymes examined showed increased activity in a dose-related fashion in both sexes. Kidneys were less responsive to treatment, however 7-ethoxycumarindeethylase activity was elevated appreciably over control levels in 600 mg/kg/day males and females. Investigators concluded that no sex-related differences in metabolic activity could explain the male-specific nephropathy in rats. Aldous, 12/22/93.

GENERAL TOXICITY INFORMATION ON VARIOUS CHLOROBENZENES

015 050543 "Technical Report. Worldwide Literature Search on Chlorobenzenes" (Tracor Jitco, Inc., 3/4/76) Partial copy of a survey of the toxicology literature for monochlorobenzene, orthodichlorobenzene and paradichlorobenzene. Acute studies show paradichlorobenzene generally least toxic. Various effects in subchronic and chronic studies were similar with all three compounds and included liver, stomach and brain toxicity. Davis 10/22/87. One-liner only.

022 064728 "Investigation of 2,5-Dichlorophenol For Clastogenic Effects in Mice Using the Micronucleus Test," (Bayer AG Institute of Toxicology, 10/6/86). 2,5-Dichlorophenol (purity = 98%), a principal metabolite of paradichlorobenzene was administered by gavage to Bor:NMRI (SPF Han) mice (10/sex/group) at 0 (vehicle = corn oil) and 1500 mg/kg with work-up (animal sacrifice) times at 24, 48 and 72 hours post-administration. The positive control was 20 mg/kg cyclophosphamide. **No adverse effect indicated.** The treated animals showed lasting symptoms of toxicity after the administration. 6 of 40 animals treated with the test compound died by the end of the test. Erythrocyte formation, as measured by the ratio of polychromatic to normochromatic erythrocytes, was also reduced. No evidence of a clastogenic effect was observed. **This study is supplementary.** M. Silva, 8/23/88.

022 064729 "Toxicology Forum," Aspen, Colorado. This record contains information on halogenated hydrocarbons and their different toxic effects, primarily related to nephrotoxicity. No worksheet. M. Silva, 8/23/88.

022 064734 "Influence of Cytotoxicity on the Induction of Tumors," Banbury Report, 25: Nongenotoxic Mechanisms in Carcinogenesis (Cold Spring Harbor Laboratory (no page or volume #, galley proofs only), 3/87. The objective of the paper was to explore several aspects of tumorigenesis including the influence of age on spontaneous neoplasia and possible roles of increased cell proliferation on spontaneous and induced neoplasia, and to place data from such studies in perspective for regulatory decision-making. Information was presented to support cytotoxicity and the resulting cell proliferation as a mechanism of tumorigenesis. This mechanism is proposed as both a promoting factor and a possible initiator in the absence of DNA damage by an agent under investigation. **This information is supplemental.** M. Silva, 8/24/88.

PHARMACOKINETIC STUDIES

Most studies in this category are part of a series of reports submitted by Monsanto, and reviewed by P. Leung, as follows:

Pharmacokinetic Study of 1,4-dichlorobenzene (p-DCB) in the F344 Rat and B6C3F1 Mouse Following Inhalation and Oral Administration

024; 98467; "Pharmacokinetic Study of 1,4-dichlorobenzene (p-DCB) in the F344 Rat and B6C3F1 Mouse Following Inhalation and Oral Administration (Blood Kinetics)" (Monsanto Company Environmental Health Lab.,St. Louis, MO, Study # EHL 88083, 11/9/90); 851; nonlabeled p-DCB added to [14C]-1,4-dichlorobenzene (p-DCB) in emulphor and ethanol (1:1) or corn oil (mean specific activity = 4.56 x 106 dpm/mg) for intravenous or oral administration, respectively; 216 mg/kg to 3 male rats/route; following intravenous administration, blood levels of p-DCB-derived radioactivity increased rapidly to maximum value of 422 ppm at 0.5 hours; however, blood levels of radioactivity following oral dosing was reported to be significantly lower (31 ppm) at this time and peak blood levels (44 ppm) were observed at 1 hour after dosing; blood levels of p-DCB-derived radioactivity decrease biexponentially irrespective of the route of administration; elimination half-life was estimated to be 16 and 11 hours after iv or oral dosing, respectively; **supplemental**; (Leung, 12/5/91).

024; 98467; "Pharmacokinetic Study of 1,4-dichlorobenzene (p-DCB) in the F344 Rat and B6C3F1 Mouse Following Inhalation and Oral Administration (Single and Multiple Oral Dosing - Rat)" (Monsanto Company Environmental Health Lab.,St. Louis, MO, Study # EHL 88083, 11/9/90); 851; 14C- 1,4-dichlorobenzene (p-DCB) in corn oil (specific activity = 2.60 - 7.36 x 106 dpm/mg); oral; single (149 or 305 mg/kg) and multiple (daily oral doses of 318 mg unlabeled p-DCB /kg/day for 13 days prior to receiving a single dose of 309 mg 14C-p-DCB/kg on day 14); 15 - 20 male rats/dose; p-DCB rapidly eliminated with a majority of the dose being excreted by 24 hours after single or multiple oral administration; at 7 days, 56 to 67% of the dose was excreted in urine and 8 to 12.5% in feces; expired air accounted for 12.4% of the dose; elimination kinetics were similar between single and multiple dose regiments; whole-body autoradiography indicated localization of radiolabeling in kidney, liver, lung, and fat; accumulation in fat and muscle after multiple dosing; 2,5-dichlorophenol and its sulfate and glucuronide conjugates were detected in urine; **supplemental**; (Leung, 12/6/91).

024; 98467; "Pharmacokinetic Study of 1,4-dichlorobenzene (p-DCB) in the F344 Rat and B6C3F1 Mouse Following Inhalation and Oral Administration (Single and Multiple Inhalation Dosing - Rat)" (Monsanto Company Environmental Health Lab.,St. Louis, MO, Study # EHL 88083, 11/9/90); 851; nonlabeled p-DCB added to 14C-1,4-dichlorobenzene (p-DCB) in methylene chloride (specific activity = 0.76 - 4.81 x 106 dpm/mg); single (male: 455 or 645 mg/kg; female: 308 or 678 mg/kg) and multiple inhalation exposures (male only: daily exposures of 921 mg unlabeled p-DCB /kg/day for 13 days prior to receiving a single dose of 871 mg 14C-p-DCB/kg on day 14); 6- hr, nose only exposure; 12-14 rats/dose; regardless of sex, 23 - 32% of the dose was excreted in urine and 2 - 3% in feces after 7 days of administration; males receiving multiple doses showed significant decreases in total recovery of dose over 7 days with 18% and 2% in urine and feces, respectively; whole-body autoradiography demonstrated that single and multiple dosing made little difference in the sites of localization (kidney, fat, intestines, and nasolacrimal duct); multiple inhalation exposures had no significant effect on the levels of glucuronide or sulfate conjugates in the urine; **supplemental**; (Leung, 12/10/91).

024; 98467; "Pharmacokinetic Study of 1,4-dichlorobenzene (p-DCB) in the F344 Rat and B6C3F1 Mouse Following Inhalation and Oral Administration (Single Oral Dosing - Mouse)" (Monsanto Company Environmental Health Lab., St. Louis, MO, Study # EHL 88083, 11/9/90); 851; nonlabeled p-DCB added to [14C]-p-DCB in corn oil (specific activity = 1.96 - 3.39 106 dpm/mg); oral; 310 or 638 mg/kg; 12-17 mice/dose; majority of the total administered radioactivity eliminated within 24 hours after dosing; urine elimination t1/2 was reported to be 2.2 and 3.2 days for the low and high doses, respectively; at 7 days 61.1 - 72.4% was excreted in urine and 11.11 - 15.74% in feces; expired air accounted for 9.1% of the administered dose; whole-body autoradiography indicated localization of radiolabeling in kidney, fat, nasolacrimal duct, stomach and gut contents; 2,5 dichlorophenol, sulfate and glucuronide conjugates were detected in urine; **supplemental**; (Leung, 12/10/91)

024; 98467; "Pharmacokinetic Study of 1,4-dichlorobenzene (p-DCB) in the F344 Rat and B6C3F1 Mouse Following Inhalation and Oral Administration (Single Inhalation Dosing - Mouse)" (Monsanto Company Environmental Health Lab.,St. Louis, MO, Study # EHL 88083, 11/9/90); 851; nonlabeled p-DCB added to 14C-1,4-dichlorobenzene (p-DCB) in methylene chloride (specific activity = 2.07 - 7.35 x 106 dpm/mg); 631 or 1240 mg/kg; 12-14 male rats/dose; 6-hr nose only exposure; urine is major route of elimination; at 7 days, 32.4 and 47.8% of the low and high dose, respectively, was excreted in urine; radioactivity in feces accounted for 19.2 and 6.1% of the low and high dose, respectively; whole-body autoradiography indicated localization of radioactivity in kidney, liver, intestines, and nasolacrimal duct; sulfate conjugates in urine accounted for about 15% of the dose in both groups, whereas glucuronide conjugates ranged from 21% at the low dose to 29% at the high dose; 2,5-dichlorophenol was also detected in urine; **supplemental**; (Leung, 12/11/91).

Summary:

Para-dichlorobenzene is rapidly absorbed following intravenous administration and a maximum blood concentration (422 ppm, Cmax) was achieved at 0.5 hour (Tmax) after dosing. However, absorption after oral dosing was slower and less extensive as evident by a smaller Cmax (44 ppm) at a later Tmax (1 hour). Renal excretion via urine is the major route of elimination with half-life of 16 and 11 hours after iv and oral dosing, respectively. By 7 days after oral dosing, 56 - 67% of the dose was excreted in urine and 8 - 12.5% in feces. Expired air accounted for about 12.4% of the dose. Elimination kinetics were similar between single and multiple oral dosing. In both the rat and mouse, significantly lower dose is available following inhalation exposure as compared to oral administration. This is illustrated by the lower amounts of the administered radioactivity recovered in the urine, feces, cage washes, carcass and tissues 7 days after single and multiple inhalation exposures (rat: 30-40%, mouse: 54-60%) as compared to oral administration (rat: 82-99%, mouse: 91-95%).

Highest concentration of the radiolabeling was found in fat in rats and mice at 6 hours after dosing and decline thereafter over a 7-day period. The amount of radioactivity was lower in fat after multiple inhalation exposures than after single inhalation exposure. After multiple oral dosing, tissue concentrations were generally higher. However, tissue concentration in fat after multiple oral dosing was lower than after a single oral dose. Whole-body autoradiography indicate the presence of radiolabeling in other tissues including kidney, liver, lung, stomach and intestines.

Comparison of male and female rats exposed to p-DCB via the inhalation route indicate minor differences in the location of the radiolabeling within the kidney. Higher levels of radioactivity were observed in the cortex of the kidney from male rats whereas in female rats and mice, higher levels of the radiolabel were found in the renal medulla. At both dose levels the sulfate conjugate was the predominant metabolite for both sexes. The total levels of sulfate and glucuronide conjugates were similar for males and females at both low and high dose levels.

Tissue clearance kinetics were generally similar regardless of dose level and route of administration. However, there were differences in kidney elimination kinetics in male rats depending on the route of exposure. In male rats dosed orally, kidney elimination was monoexponential with an apparent half life of 1.4 days. However, the inhalation exposed rats showed that kidney elimination occurred biexponentially with a half-life of 4.7 days, similar to other tissues. Furthermore, male mice exhibited biexponential clearance kinetics following oral and inhalation dosing.
Total recovery of p-DCB-derived radioactivity was the same for rats and mice 7 days after oral administration (305 and 310 mg/kg, respectively) or inhalation exposure (645 and 631 mg/kg, respectively). In general, mice exhibited a more rapid rate of elimination from urine when compared to rats as indicated by the elimination half-lives following oral administration (2.2 vs. 9.7 days). Higher levels of radioactivity were detected in urine 7 days after oral administration. On the other hand, radioactivity levels in the feces were higher in mice following inhalation exposure throughout the 7 day collection period.

Data from this study demonstrated that oxidation of p-DCB to 2,5- dichlorophenol followed by conjugation to glucuronide and sulfate represents the major pathway of biotransformation.

50395-041 138889 (relates to 50395-024:098467 pharmacokinetics studies). [EHL 88246] Wilson, A.G.E., R.A. Freeman, and C.M. Reisch, "Development of physiologically-based pharmacokinetic models for 1,4-dichlorobenzene (p-DCB) in the rat", Monsanto Co. (EHL), 5/16/95. Study takes data, largely from Record No. 098467, and applies data to **(1)** a "two compartmental model" (using observed levels in blood and individual tissues over time), **(2)** a "total radioactivity distribution model" (using a PB-PK approach to follow distribution of label in different tissues over time), **(3)** a "p-DCB distribution model" (similar to total radioactivity model, but restricted to p-DCB), or to **(4)** a "multi-metabolite model" (similar to the last two models, but designed to track p-DCB and selected major metabolites, specifically 2,5-dichlorophenol and the sulfate and glucuronide metabolites). Plotted simulations and simulated output tables are provided for various models. Annotated program codes are also provided, including constants for fractional blood flow, partition coefficients, and other factors. Appendix II is a report of *in vitro* experiments using rat and mouse hepatocyte and liver microsomal preparations to compare metabolic patterns. Collective data may be useful for predicting compartmental exposures over time and for interspecies comparisons. Aldous, 4/11/96.

OTHER STUDIES ON THE METABOLIC DISPOSITION OF CHLOROBENZENES

50395-033 126847 Schmidt, U., "Concentration measurements of 1,4 dichlorobenzene and 2,5 dichlorophenol in the plasma, urine, and the fabric [sic] of fat and liver, of rats", Bayer AG, Institut f~~u~~r Toxikologie, Wuppertal, Dec. 4, 1978. Five rats/sex/group per sacrifice interval (age and strain unspecified) were dosed 5 hr/day, 5 days/wk by inhalation for up to 18 months at 0, 450, or 3000 mg/m3 nominal exposure levels. Sacrifices were done at month 6, month 18, and

6 months after cessation of dosing following 18 months of treatment. Urine and plasma levels, as well as fat and liver levels of para-dichlorobenzene [1,4-DCB] and 2,5 dichlorophenol [2,5-DCP] were measured at each interval. Samples were treated with sulfatase and glucuronidase prior to assay. On a mg/g or mg/ml basis, the lowest to the highest residues were in liver, plasma, urine, and fat, respectively. There was no obvious sex difference. Urine contained exclusively 2,5-DCP, plasma contained predominantly 2,5-DCP, liver contained predominantly 1,4-DCB, and fat contained entirely 1,4-DCB. Tissue levels were reported to be much lower generally at 18 months than at 6 months. After 6 months cessation of treatment, no residues were detected in fat. This is a supplementary study of limited qualitative value. Aldous, 12/27/93.